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THE EXPERIMENTAL PRODUCTION OF DEAFNESS IN YOUNG ANIMALS BY DIET

By EDWARD MELLANBY

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In a series of publications since 1926 it has been shown that degenerative changes in many nerves of both central and peripheral systems of young animals can be readily produced by dietetic means [Mellanby, 1926, 1930, 1931, 1933, 1934*b*, 1935, 1937, 1938]. The most common lesions consist of demyelination changes followed by complete disappearance of the fibres in many of the afferent peripheral nerves and ascending fibres in the central nervous system. It has been shown that nerve cells are also affected by these dietetic conditions and reasons have been given which suggest that these nerve cell changes may be primary to the demyelination of the nerve fibres.

In the usual experimental period of about four months, the afferent fibres degenerate before the efferent, which may not be much affected, if at all. With a longer dietary period the efferent fibres are affected but never as badly as the afferent.

Although the most obvious lesions consist of changes in the myelin sheath there are also changes in the axis cylinders in the nerves of both dogs and rabbits fed on diets deficient in carotene and vitamin A. These changes can be seen in fibres which show annular myelin degeneration and even in cases where there is no other obvious defect in the nerve. The axis cylinder changes have been studied in more detail in branches of the sensory nerves supplying the dental tissues by King, Lewinsky & Stewart [1938] in vitamin A-deficient rats.

The dietetic conditions favouring these developments are (1) deficiency of vitamin A and carotene, and (2) presence of much cereal. Of these two factors, the absence of vitamin A and carotene is the more important, for in young rats at least, the same demyelination changes can be produced by synthetic diets in the absence of cereal if they are deficient in vitamin A

and carotene [Zimmerman, 1933]. Again, the cereal content of the diet may be very high and yet, in the presence of sufficient vitamin A and carotene, little or no nerve degeneration will be produced. On the other hand, changes in the type of cereal eaten or the addition of certain cereal products may alter the degree of degeneration. The experimental diets are made up of ordinary foodstuffs, except for the irradiated ergosterol, and are rich in all substances of the vitamin B complexes.

One of the afferent nerves affected by demyelination changes under these dietetic conditions is the 8th, both cochlear and vestibular divisions, the former more so than the latter. Degenerate changes in the vestibular nerves are associated with inco-ordinate movements of locomotion of the animal, as might be expected. Young dogs showing this condition are also inattentive and do not respond to the call of people at close range, a characteristic which distinguishes them from normal puppies. This inattention is doubtless due partly to deafness.

The present paper is concerned with these abnormal changes due to vitamin A deficiency, and contains a description of the pathological condition of the labyrinthine capsule, both of its nerve supply and the bone itself. When a rich source of vitamin A is added to the diet while every other condition of diet and environment is kept constant, the animals remain normal in behaviour and the labyrinthine capsule escapes pathological change.

All young animals examined up to date, i.e. dogs, rabbits and rats, react to the above dietetic defects, so far as nerve degeneration is concerned, in the same way, differing of course in degree. The new facts, especially concerning bone hyperplasia, described in this paper, refer for the time being to the dog. There is, however, no doubt that bone hyperplasia takes place in the rabbit and probably in the rat, and will be described in a future paper.

Histological technique

Since the degenerative changes of a nutritional nature in the 8th nerve were first seen and described, the need of following up and examining in detail the internal ear itself has been constantly in mind. Many of the animals fed over a fairly long period on diets very deficient in vitamin A were obviously deaf, and unsuccessful attempts were made to grade the degree of deafness in dogs and rabbits by observing their reactions to sound. The earlier histological preparations made for the detailed examination of the finer structure of the cochlea and vestibule were unsatisfactory. Last year I discussed the subject of deafness in animals on

deficient diets with Mr Cleminson, Head of the Ferens Institute for the Study of Otology, and he was good enough to arrange for a demonstration of the technical methods for histological examination of the internal ear in use at that Institute. Mr Peet kindly demonstrated these methods of fixation by perfusion through the heart and gave full details of the further technique. Moreover, serial sections of celloidin embedded material were made from two labyrinths fixed by Mr Peet. These and other sections were carefully examined by Dr Hallpike, to whom I am most grateful for assistance and advice. The Ferens technique has in the main been used in the present work, with the exception that, in order to shorten the time needed for obtaining the final result, paraffin has replaced celloidin embedding in the end process. In many cases formol-saline was used as a fixative agent instead of Wittmaack's solution which contains potassium bichromate. For decalcification 5 % nitric acid was often used instead of 1 %. By these devices the time occupied in preparation was reduced from 4 to 5 months to between 5 and 7 weeks. The resulting histological preparations are not as good as those obtained by the Ferens Institute method, especially as regards nerve cells and epithelial structure, but the great saving of time counterbalances this drawback. As I understand that Dr Hallpike will publish a detailed account of the Ferens Institute method for the histological study of the labyrinth capsule, no further reference will be made to the methods used in the present work.

A total of fifty-one labyrinths from 44 dogs have been examined. Serial sections of sixteen of these from twelve animals have been cut, stained and examined; in some cases when both labyrinths of one animal were examined, pathological changes were not identical. The whole cochlea and vestibule of the remaining cases were cut but not mounted serially. In addition, ground undecalcified sections were prepared from some of the ears for purposes of bone examination. Some experiments of a similar nature have been made also on rabbits and rats and serial sections of the labyrinthine capsules cut. These experiments will not be described in the present publication. It is now evident that because of the complicated structure of this organ and the multiple changes that occur in the labyrinth under the experimental conditions that it would have been easier if serial sections had been made from all the experimental animals, in order to appreciate more fully the actual course of events. This, however, was not possible, but the number of labyrinths serially sectioned is probably sufficient to allow of a general description of the changes produced.

Experimental methods and results

The experiments on young dogs described in the present paper were not made primarily to test the effects of different diets on the ear. The action of certain forms of diet in producing and preventing extensive nerve degeneration had been well established and most of the experimental feeding was at the time centred on an attempt to get information on the part played by cereals and cereal products in influencing these changes. This explains why, so far as the cereal portion of the diet is concerned, there are small differences in the various series of experiments. It is not the intention in the present publication to deal with this point, however, since the effect of changing the cereal portion of the diet is only one of degree and not of kind. For the time being attention will be mostly confined to the changes in the neurones and bones of the cochlea and labyrinth produced by variations in the vitamin A content of diets rich in cereals. Reference will, however, be made to some animals in whose diet potato wholly replaced the cereal.

The experiments were made under the following conditions.

Litters of puppies varying from 7 to 10 weeks old were given diets consisting of separated milk, cereal (oatmeal, white flour etc.), lean meat, yeast, peanut oil, irradiated ergosterol (vitamin D), orange or lemon juice, and sodium chloride. In this diet there is very little vitamin A or carotene, but it is, as far as is known, otherwise complete.

The time necessary for producing the pathological change varies with the stores of vitamin A in the liver of the puppy at the beginning of the experiment and also with the severity of the dietetic deficiency. Usually, however, the animal's behaviour is abnormal after 2–4 months of such feeding and the changes may be great after 4 months. In the experiments reported in this publication the duration of the dietetic period varied from 4 to 10 months in different animals. The pathological changes were very intense in the dogs which had received vitamin A-deficient diets for the longer periods.

Apart from the specific effects produced by the vitamin A deficiency, the animal's health was usually fairly good, the food was eaten well and the weight steadily increased. Dogs do not readily die of infection in these conditions, as they might be expected to do when one thinks of the multiple infective lesions developed by young rats fed on vitamin A-deficient diets. Nor do dogs develop xerophthalmia as easily as do rats but, when they do, it is a more serious matter, owing to the rapidity with which perforation of the cornea often takes place. The relation of xero-

phthalmia to degeneration of the trigeminal nerve has been previously described [Mellanby, 1934*a*].

A typical experiment in which advanced degenerative changes were produced will be given first. Three puppies aged 8 weeks were given the following dietary mixture daily over a period of 42 weeks: separated milk powder 12·5 up to 30 g., oatmeal 75 up to 270 g., peanut oil 7·5 up to 15 c.c., meat deprived of visible fat 25 up to 60 g., baker's yeast 3·75 up to 12 g., sodium chloride 1 up to 4 g., vitamin D as irradiated ergosterol 1000 I.U. The former quantity represents the amount of each constituent given at the beginning of the experiment and the latter the amount given when a maximum diet was being eaten. In addition, dog I had daily 30,000 I.U. of vitamin A. Dog II did not get any additional source of vitamin A throughout the feeding period. Dog III received no added vitamin A for the first 15 weeks, but for the last 27 weeks it had 30,000 I.U. daily.

At the end of the experiment the vitamin A content, measured as Carr-Price blue values using a Lovibund tintometer, of the livers of the three dogs was as follows:

Dog	Carr-Price blue values per g. liver	Approx. equivalent in I.U. per g. liver
I (vitamin A-rich diet)	93	2800
II (vitamin A-deficient diet)	Nil	Nil
III (curative experiment)	50	1500

Dog III will not be further discussed here, but will be referred to later in dealing with possible recovery.

Fig. 1 represents a section of the cochlea and its components of dog I (vitamin A-rich diet). Fig. 2 is a corresponding photomicrograph of dog II (no vitamin A). (Only a few of the photomicrographs illustrative of the results obtained were made from dogs I and II. Other illustrations were sometimes chosen, not because they were different, but because they were technically better.)

The most obvious pathological changes found in the labyrinths of young dogs brought up on these vitamin A-deficient diets are (*a*) nerve degeneration, more especially of the cochlear neurones, (*b*) new bony growth in the modiolus, (*c*) overgrowth of the internal periosteal layer of the capsule, i.e. the bone in proximity to the brain, (*d*) serous labyrinthitis, (*e*) degenerative changes of the organ of Corti and sensory epithelium of the semicircular canals. Occasionally a small amount of bony overgrowth was seen in the basal whorl of the scala tympani.

Changes in the 8th nerve (*cochlear and vestibular divisions*). The cochlear division (or its remains) of the 8th nerve is seen in Figs. 1 and 2.

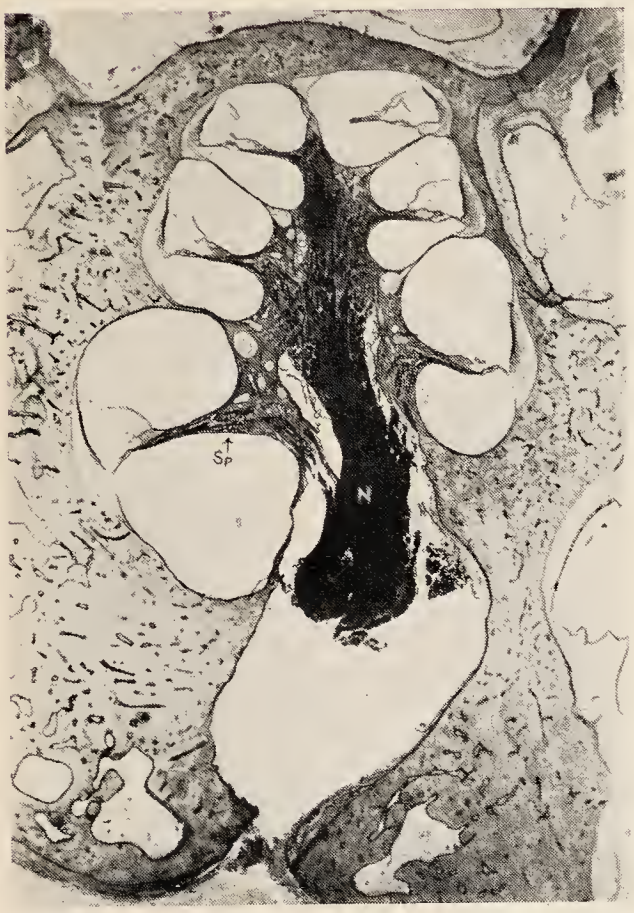

Fig. 1.

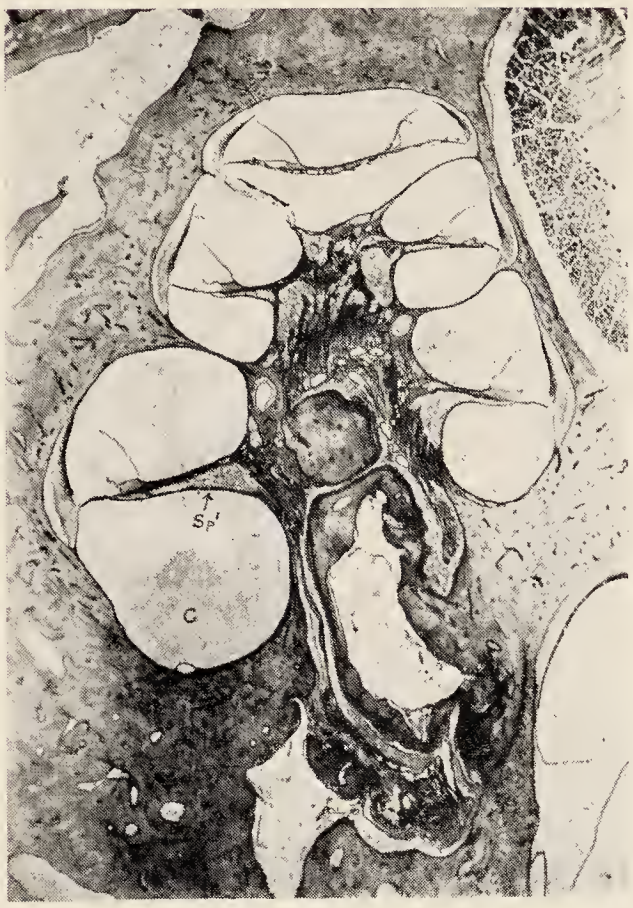

Fig. 2.

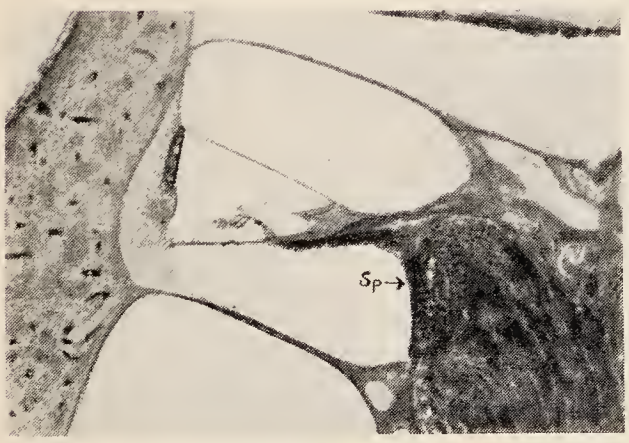

Fig. 3.

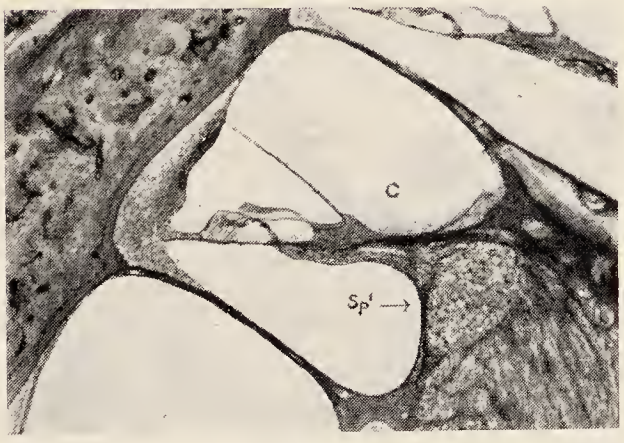

Fig. 4.

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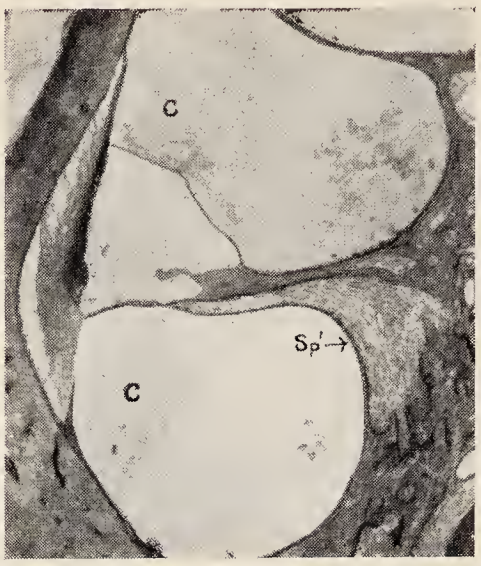


Fig. 5.

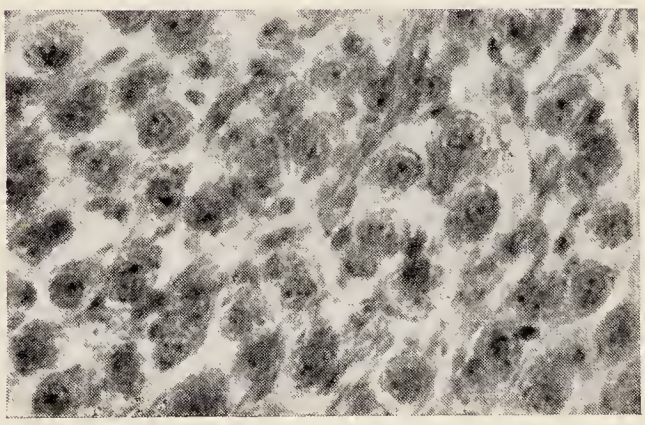


Fig. 6.

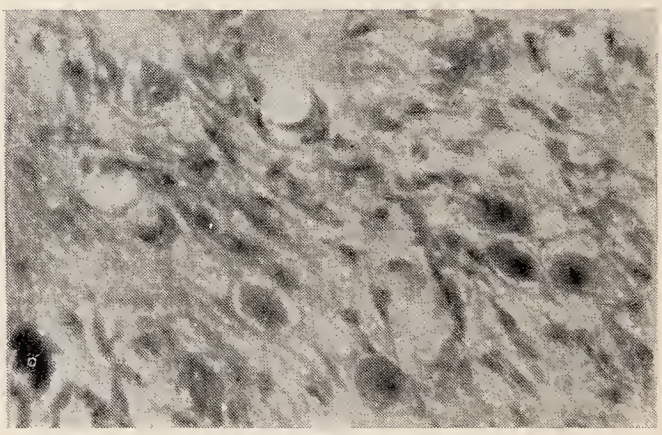


Fig. 7.

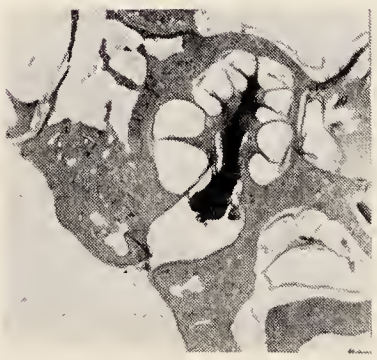


Fig. 8

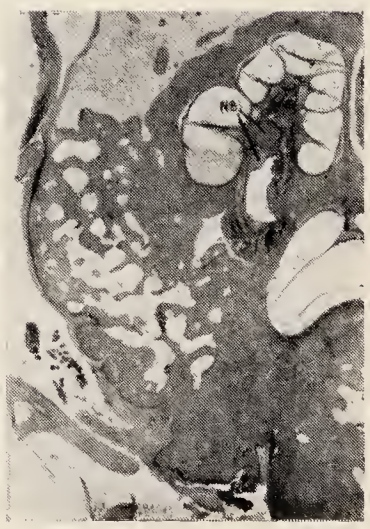


Fig. 9*a*.

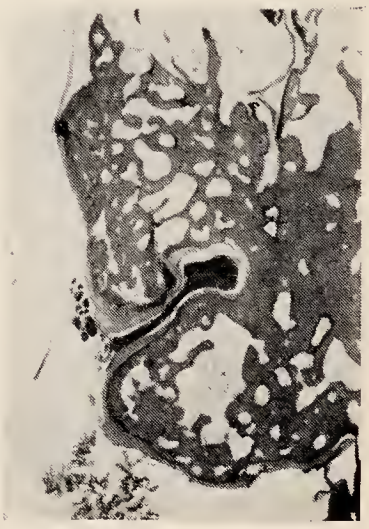


Fig. 9*b*.

In Fig. 2, representing the cochlea of dog II (vitamin A-deficient diet for 10 months), this nerve has disappeared, whereas in Fig. 1 from dog I (vitamin A-rich diet) it is intact. Not only has the cochlear nerve of dog II completely degenerated but the spiral ganglion cells and the nerve fibres to the organ of Corti have gone, and their place is filled with loose connective tissue. The spiral ganglion cells and nerve fibres to the organ of Corti of dog I are normal.

Photomicrographs ($\times 50$) of a whorl of a cochlea of each of three dogs are seen in Figs. 3–5. Fig. 3 is normal and is in great contrast to both Figs. 4 and 5, which are from dogs fed on diets deficient in vitamin A. In Fig. 4 the vitamin A-deficient animal was killed after 5 months of the diet. Many cells of the spiral ganglion have disappeared and those remaining are shrunken and the cytoplasm is free from granules. Most of the peripheral branches to the organ of Corti have also gone. In Fig. 5, taken from dog II, which had been on the vitamin A-deficient diet 10 months, the condition is still worse; there are no cells and no fibres left. Figs. 6 and 7 are high-power photomicrographs ($\times 500$) showing the detailed condition of the cells of the spiral ganglion in a normal (receiving vitamin A) and a vitamin A-deficient animal respectively. The normal spiral ganglion cells seen in Fig. 6 are very different from those in Fig. 7, which represent part of the spiral ganglion seen in Fig. 4. Comparison between Figs. 3–5 also shows that in the vitamin A-deficient animals (Figs. 4, 5) the peripheral fibres of the cochlear nerve supplying the organ of Corti have degenerated, whereas in the animal receiving vitamin A (Fig. 3) they are present.

It will thus be seen that in an advanced case (Fig. 5) the whole cochlear nerve, including the spiral ganglion cells and their central and peripheral branches, disappears.

In experiments where the nerve degeneration is not as advanced as in the instance just described, the spiral ganglion cells at the base of the helix are usually rather more abnormal than those at the apex; one exception to this rule, where the basal spiral ganglion cells are less affected than the apical cells, was encountered.

Although all the cells of the spiral ganglion of dog II (Fig. 2) were degenerated, there were still some cells in Scarpa's ganglion. It is true that they were in most cases degenerate but they had not completely disappeared as had the cells of the corresponding spiral ganglion. Throughout this investigation it has been found that the cochlear division of the 8th nerve is more affected than the vestibular. The ganglion cells of Scarpa's ganglion in dog I (vitamin A-rich diet) are normal. These results

are in keeping with those previously described [Mellanby, 1935], where it was shown that, comparing the number of degenerating fibres in the cochlear and vestibular divisions of the 8th nerve in A-deficient animals, it was always found that the cochlear division suffered more severely than the vestibular division.

Overgrowth of bone of the labyrinthine capsule. A glance at Figs. 1 and 2 shows another striking development in the internal auditory meatus of the vitamin A-deficient dog (Fig. 2). It will be seen that two massive pieces of bone fill up the meatus and leave little or no space for the branch of the 8th nerve. The bone appears on the whole to be of normal structure but the lower piece has a large cavity full of fatty tissue. The corresponding meatus of Fig. 1 (vitamin A-rich diet) is patent and the 8th nerve here has an uninterrupted passage to the brain.

The filling of the internal auditory meatus at the modiolus end seen in Fig. 2 is only found in advanced cases of degeneration produced by the prolonged dietetic deficiency described, but all experimental animals, so far examined, brought up for a few months on similar diets show some such bony change.

Another position where bony overgrowth is found is in the periosteal bone adjacent to the brain and to the internal auditory meatus. This new bone can be seen by comparing Figs. 8, 9*a* and 9*b*, which are low power photographs of the labyrinthine capsule of dog I (Fig. 8) and dog II (Figs. 9*a* and 9*b*). In Fig. 8 (diet rich in vitamin A) the meatus and the cochlear division of the 8th nerve can be traced from the helix of the cochlea to the edge of the capsule. In Fig. 9*a* the meatus is tortuous and the exit of the nerve from the brain cannot be seen, so that several sections are needed to trace its full course. This displacement is illustrated in Figs. 9*a* and 9*b*; Fig. 9*b*, where the exit of the 8th nerve or its remains is clearly seen, represents a section about 2·5 mm. from that shown in Fig. 9*a*.

The depth of bone between the basal whorl of the cochlear helix and the brain is much greater in Fig. 9*a* (from A-deficient animal) than in the control (Fig. 8). The cancellous nature of the new bone is also clearly seen in Figs. 9*a* and 9*b*. This increase in periosteal bone is more clearly demonstrated in Figs. 10 and 11 representing sections cut in a plane at right angles to those of Figs. 8 and 9*a* and 9*b*. It will be seen that whereas in the normal dog (Fig. 10) the bone on each side of the internal auditory meatus is thin, in Fig. 11 it is greatly thickened, due to the laying down of new periosteal bone, which appears to be of the ordinary cancellous type. There is no excess of osteoid tissue, nor is there any evidence of the

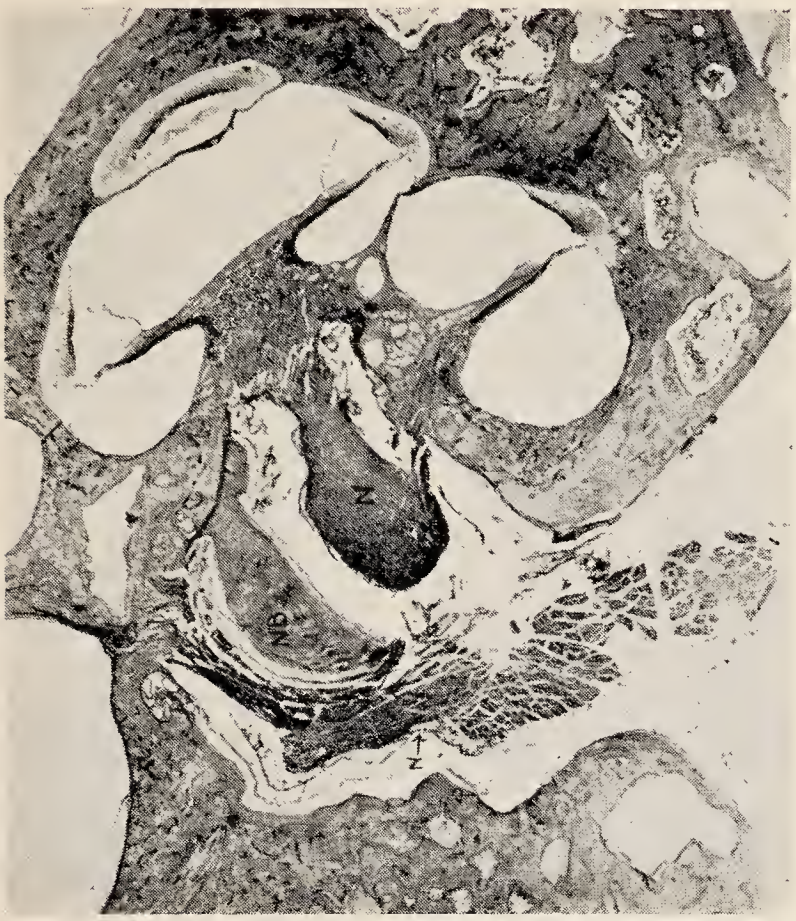


Fig. 12*b*.

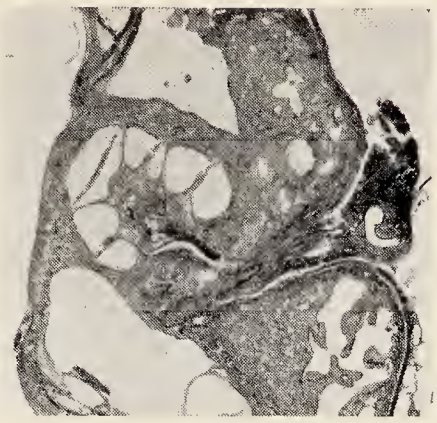
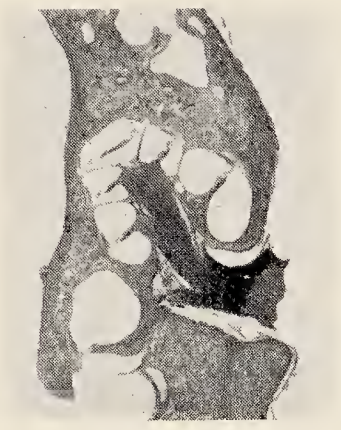


Fig. 11.



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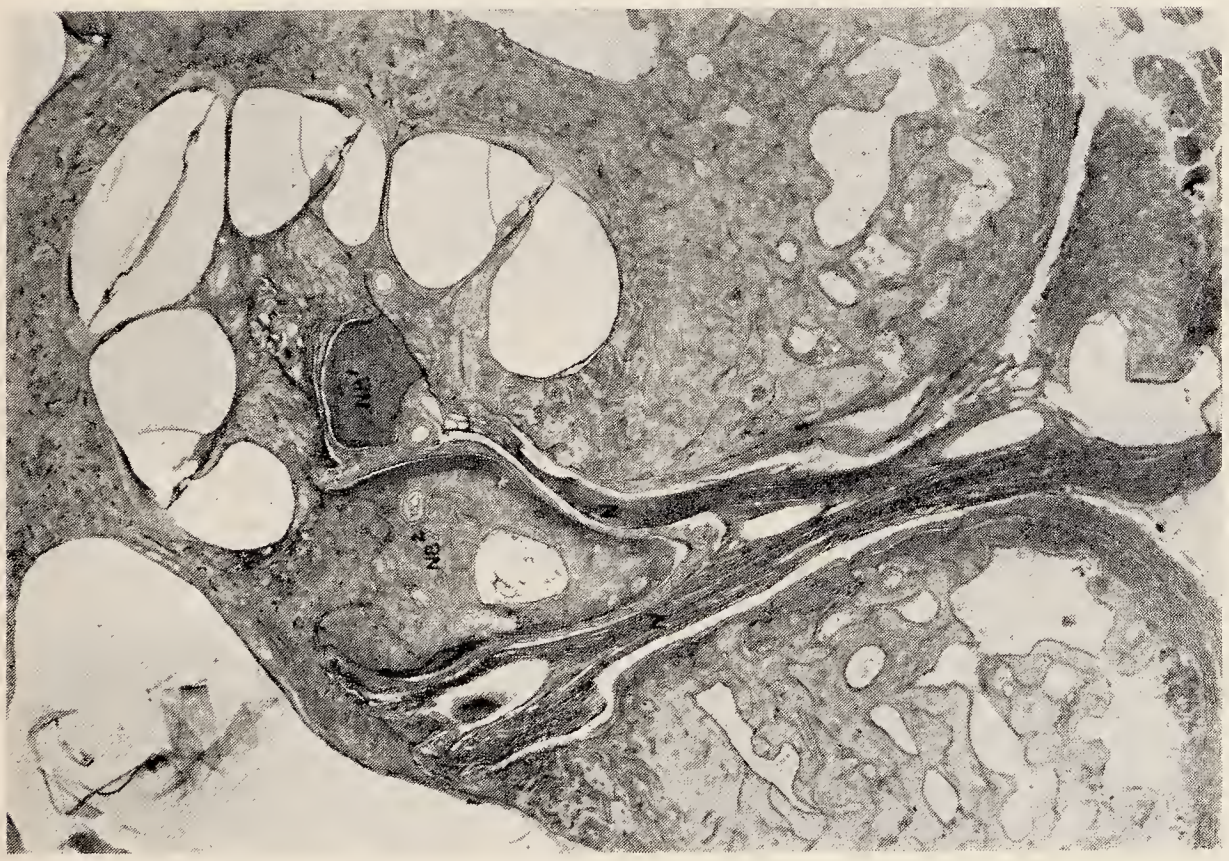

Fig. 12*a*.

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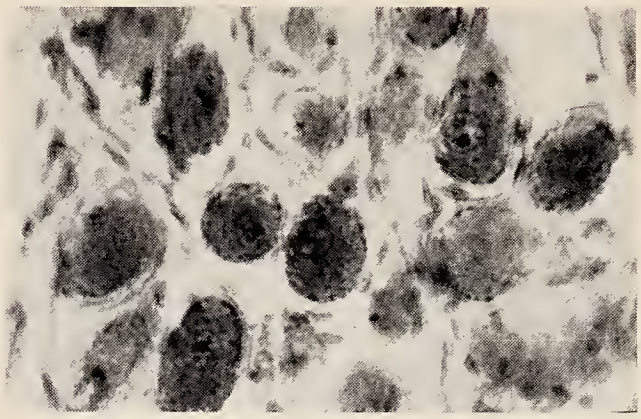

Fig. 13.

Fig. 14.

Fig. 15*a*.

Fig. 16*a*.

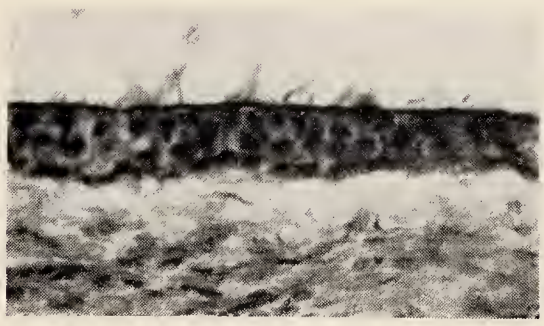

Fig. 15*b*.

Fig. 16*b*.

changes being inflammatory in origin. The spaces in the bone are abundant in the outer periosteal layer and are usually filled with fat.

The effect on the nerves of these two bony overgrowths, the one in the modiolus filling the upper part of the internal auditory meatus, and the second resulting in the thickening of the periosteal layer must be harmful. The first squeezes the nerve; the second greatly lengthens the distance from the respective ganglia of the cochlear and vestibular division of the 8th nerve and thus stretches the nerve.

Compression not only of the cochlear but also of the vestibular division of the 8th nerve by new bone formed in and near the modiolus can be well seen in Fig. 12*a*, which represents the cochlea of a dog after 5 months on a vitamin A-deficient diet. In this figure a smaller mass of new bone (NB^1) is present in the modiolus above the spiral ganglion at the base of the helix and a second larger mass (NB^2) between the division of the cochlear and vestibular branches of the main nerve. The spiral ganglion cells have largely degenerated (see Fig. 7 and compare with the normal in Fig. 6), but in the case of the cells of Scarpa's ganglion, although many of these are squeezed into an abnormal shape, they have not degenerated (see Fig. 14 and compare with the normal in Fig. 13).

It has been mentioned above and elsewhere [Mellanby, 1935] that the cochlear division of the 8th nerve is more liable to be completely destroyed by these nutritional conditions than the vestibular division, and yet both branches are compressed and stretched by these bony overgrowths. This possible difference in susceptibility to mechanical influences will be considered later.

Serous labyrinthitis. Besides the nerve and bone changes described above, the condition known as serous labyrinthitis was also produced by these diets deficient in vitamin A. The albuminoid coagula in the perilymph spaces which are characteristic of this condition can be seen in Figs. 4 and 5. In the normal animal (Fig. 3) no such coagula can be seen. A similar pathological condition is well known in man. There is no evidence in the present experiments that the process is infective in origin, nor indeed that any of the changes described is the result of inflammation.

What the relation is between the serous labyrinthitis in the vitamin A-deficient animals and the nerve and bone changes cannot yet be stated. It is possible that the abnormality of the perilymph is due to the spread of some toxic influence from the sub-arachnoid space of the posterior fossa up the cochlear aqueduct, as this is a common pathway for the transit of toxins in serous labyrinthitis in man [Hallpike, 1937]. This point i being further investigated.

Serous labyrinthitis in man is also associated with a series of degenerative changes in the organ of Corti and the sensory epithelium of the vestibule. Similar changes take place in the animals under discussion.

Degenerative changes in the organ of Corti. In serous labyrinthitis in man, one of the earliest changes in the organ of Corti is the loss of differential staining power between cytoplasm and nuclei in the cells of the rods of Corti and in the outer and inner hair cells. This change cannot be seen in the histological preparations so far made in the present work, probably because the technique used is not suitable. In severer cases of experimentally produced serous labyrinthitis, the degeneration of these cells becomes more marked, the hair cells lose their hairy projections and ultimately these auditory cells, as well as Deiter's and other cells of the organ of Corti disappear, leaving a small amount of undifferentiated tissue lying on the basement membrane (Fig. 5). In spite of the degeneration of these cells, there still remains the small tunnel of Corti. In Fig. 3, representing the organ of Corti of a dog receiving vitamin A, the structure is normal.

Degenerative changes in the epithelial cells of the ampullae. Up to the present time a comparative study of the vestibular apparatus has not been made but changes in the epithelium of the semicircular canals in those animals fed for long periods on A-deficient diets are also obvious. Comparing Figs. 16*a* and 16*b* (vitamin A deficient) with Figs. 15*a* and 15*b* (receiving vitamin A), it will be seen that there is a general thickening of the epithelium in the former. Associated with this thickening there is a loss of differentiation of the individual cells, the disappearance of the nuclei and a shedding of hairy projections.

Relation of serous labyrinthitis to end-organ degeneration. When the degenerative changes in the organ of Corti and in the sensory epithelium of the vestibule above described were first noted, it was thought that these might be due to degeneration of the cochlear and vestibular nerves respectively. Dr Hallpike informed me, however, that this was not the case, since there was good evidence that cutting the 8th nerve, although it produced degeneration of the whole cochlear neurone, did not produce degenerative changes in the organ of Corti. He also informed me that serous labyrinthitis in man ultimately affected the organ of Corti and the sensory epithelium of the ampullae. It seems likely, therefore, that this is the sequence of events in the present experiments.

Serious structural changes in the organ of Corti are a late event compared with the nerve degeneration and bone hyperplasia and appear always to be accompanied by serous labyrinthitis. On the other hand, in

shorter experiments there may be serous labyrinthitis without large structural defects in the organ of Corti. There is also a suggestion from one result in the present work that serous labyrinthitis can be cured by adding vitamin A to the diet even after the bone overgrowth and nerve degeneration are advanced. It is likely, for instance, by analogy with the other experimental results that dog III (curative experiment) had developed serous labyrinthitis as well as nerve and bone changes at the end of the 15 weeks on a vitamin A-deficient diet. This dog received abundant vitamin A for the next 27 weeks and at the end of this period the cochlear neurone had almost completely degenerated. There was, however, little or no serous labyrinthitis nor was there any abnormality of the organ of Corti or sensory epithelium of the ampullae of the semicircular canals. It would appear possible from this experiment that the addition of vitamin A, after a period of vitamin A deficiency, cleared up the serous labyrinthitis and so prevented the degeneration of the organ of Corti but did not prevent the process of destruction of the cochlear neurone. This possible explanation would be in keeping with the view that degeneration of the sensory epithelium of the labyrinth in these experiments is due to serous labyrinthitis and not to degeneration of the auditory nerve. Further experiments to settle this point are, however, necessary.

Serous labyrinthitis is probably not so early a pathological development as bone overgrowth or nerve degeneration, although it is true that wherever bone hyperplasia and nerve degeneration are advanced (apart from the curative experiment of dog III) serous labyrinthitis exists. In early and slighter cases of bone and nerve abnormality there may be no serous labyrinthitis. This is best seen in experiments in which the cereal element of a vitamin A-deficient diet is replaced by potato. The amount of bone hyperplasia in such cases is comparatively small though definite, and the cells of the spiral ganglion are slightly degenerated, but there is little or no serous labyrinthitis and the cells of the end-organs are apparently normal (Fig. 12*b*).

Early changes in labyrinth due to dietetic abnormality. As some of the results, especially the bony overgrowth, were quite unexpected, the appearance in earlier stages of the abnormality has not so far been studied, although histological material is now being prepared from experiments of shorter duration.

These early changes can, however, be well seen in a series of experiments in which the cereal portion of the diet was replaced by potato. The diets otherwise were as previously described. It has been already pointed

out [Mellanby, 1934*b*] that replacement of cereal by potato in these vitamin A-deficient diets greatly reduces the degree of nerve degeneration. What may be the real explanation of this effect is not known—it is possible that cereals have a toxic action and hasten the pathological changes in the absence of vitamin A and that their replacement by potato reduces this action, or it may be that the small amount of carotene in potato is sufficient to prevent the development of the more pronounced changes. It may be added, however, that the dogs eating the potato diets had no stores of vitamin A in the liver at death. This point will not be considered further here.

After a period of 5 months on the potato diet deficient in vitamin, the animals were very active and comparatively normal in appearance and behaviour, and contrasted greatly with those on a similar diet but containing cereal. Histological examination of the labyrinthine capsule at the end of this period usually shows a small amount of bone overgrowth in the modiolus and increased periosteal bone; this can be clearly seen in Fig. 12*b*. How relatively small the bone overgrowth is can be seen if comparison is made with Fig. 12*a*, which represents the labyrinth of a dog maintained on a cereal diet deficient in vitamin A for the same period (i.e. 5 months) as the potato-fed dog of Fig. 12*b*.

The amount of nerve degeneration in this latter dog is slight but definite. There is actually only a small amount of degeneration in the cochlear neurone (central fibres) but the spiral ganglion cells appear abnormal to some extent.

Little or no serous labyrinthitis is present in these cases and no degenerative changes of the organ of Corti or the sensory epithelium of the vestibule can be seen.

Overgrowth of bone at the base of the skull obvious to the naked eye. After microscopic examination had revealed the large bony overgrowth produced by these vitamin A-deficient diets it was soon obvious, even to the naked eye, that the base of the skulls of these animals was greatly deformed by this overgrowth. In addition to the auditory nerve, it was evident at once that other nerves including the optic, trigeminal and facial were pinched by the bony overgrowth and that this mechanical pressure might well explain the degenerative changes of these nerves previously described. This matter will be considered in more detail in a subsequent publication.

Rabbits and rats. That there are also changes in rabbits and rats fed on vitamin A-deficient diets there can be no doubt, but details of the abnormalities have not been studied sufficiently to warrant a description

here. In the vitamin A-deficient animals so far examined, there are abnormalities, including nerve fibre and cell degeneration, bony overgrowth and serous labyrinthitis.

Discussion of results

Similar experiments previously published [Mellanby, 1934*b*, 1935] had shown that the central branches of the cochlear and vestibular divisions of the 8th nerve in the labyrinthine capsule were degenerated to some degree, the cochlear fibres more than the vestibular. The present work confirms this earlier work and allows a much better judgment to be made both of the degree of degeneration and the condition of the various parts of the cochlear neurone. In some cases the whole cochlear division had degenerated and the animals must have been completely deaf. In such cases the vestibular division was also severely affected but not to the same degree, and even when the cells of the spiral ganglion had completely disappeared it was usual to find some cells of Scarpa's ganglion remaining.

The surprising result of the present investigation was the new bone formation in the labyrinthine capsule which accompanied the nerve degeneration. So far as the writer knows, there is no previous evidence that vitamin A deficiency results in bone overgrowth. This new bone, so far as the labyrinth is concerned, affects the periosteal bone near the brain and is not found in the deeper layers away from the brain. It is of cancellous nature with marrow cavities usually full of fat. It is normal in appearance and is not of inflammatory origin. Indeed, the capsules of these affected dogs are apparently free from infection in the ordinary sense. This point is mentioned because it will be remembered that rats brought up on vitamin A-deficient diets often have infected middle ears [Green & Mellanby, 1928].

It has been pointed out that the new bone laid down appears constantly in two places, although in each it is of periosteal origin. The first of these positions is in and near the modiolus itself. This bone may appear more compact and usually only one or two cavities, sometimes large, are obvious in any one section. This bone can be seen in the photomicrographs to press on and elongate the nerves as they leave the cochlea and vestibule respectively. The second position of bone overgrowth is round the internal auditory meatus and adjacent to the brain. It has the effect of placing the cochlea deeper into the capsule and of increasing the distance from the spiral and Scarpa's ganglia to the brain. This must stretch the nerve. In addition, however, this bony overgrowth sometimes

twists the internal auditory meatus, so that it may be impossible to get a complete section of the nerve in one histological preparation, as can usually be done in normal animals. This twisting of the internal auditory meatus must also stretch the nerve.

New bone may also occasionally be seen in a third place, namely in the scala tympani at the basal whorl of the helix. The inconstancy of the small amount of bone in this third position indicates that it is probably independent of the other two masses of periosteal bone and may rather be related to serous labyrinthitis.

The general result of this investigation, therefore, is that a deficiency of vitamin A in the diet has resulted in the development of newly formed bone and the degeneration of the 8th nerve, especially the cochlear neurones. As a rule, the greater the bone formation, the greater the degeneration of the nerves of the labyrinth, and the conclusion that the nerve degeneration is due to mechanical interference produced by bone overgrowth is difficult to avoid. This deduction is supported by what is already known of the reaction of each division of the auditory nerve to injury of their central branches. If the cochlear nerve reacted as do most sensory nerves to injury of its central branch, it would not be expected that the cells of its spiral ganglion and their peripheral branches should be so easily destroyed by mechanical influences bearing on its central branch in the internal auditory meatus. It is known, however, that the cochlear nerve is unlike most other sensory nerves in this respect and is even unlike the vestibular division. For instance, Wittmaack [1911] showed that destruction of the central branch of the 8th nerve in the internal auditory meatus with preservation of the blood supply caused degeneration of the cells of the spiral ganglion with their peripheral branches to the organ of Corti. Wittmaack also found that the peripheral vestibular branch and Scarpa's ganglion did not degenerate after this operative procedure, although the central branches of the vestibular nerve were destroyed. These results have been confirmed by Kaida [1931], by Hallpike & Rawdon-Smith [1934] and Hallpike [1938]. Other confirmation comes from the observations of Crowe [1929], corroborated by de Kleyn & Gray [1932], that pressure upon the 8th nerve by a tumour in the internal auditory meatus leads to degeneration of the peripheral cochlear neurone.

These facts show that the degenerative reactions of the cochlear nerve represent a notable exception to Waller's law. It is clear, also, that the condition of the cochlear neurone in the present work is in keeping with its degeneration being due to pressure and stretching of the central fibres by the newly formed bony masses.

In contrast to the great susceptibility of the cochlear nerve to compression and other injuries of its central branch, the resistance of the vestibular nerve to compression and elongation, which has been demonstrated above (Figs. 4, 5), is noteworthy. The experiments of other workers have also demonstrated this fact, for cutting the central branch of the vestibular neurone, as in the case of most other afferent nerves, produces only temporary derangement of Scarpa's ganglion and no degeneration of the peripheral branch [Hallpike, 1937]. Thus the better preserved condition of the vestibular nerve in comparison with that of the cochlear nerve again supports the view that the nerve destructive changes are mechanical in origin and are due to bone overgrowth.

The point under discussion, namely, whether bone overgrowth is directly responsible for the degeneration of the 8th nerve, is of fundamental importance and obviously cannot rest at the present point. The nerve degeneration produced by these vitamin A-deficient diets is widespread and includes not only the 8th nerve but also the optic and trigeminal nerves, the posterior roots of the spinal cord and many ascending fibres in the cord, including the endogenous nerve fibres of the anterior and posterior spino-cerebellar tracts. If, therefore, bone overgrowth is responsible for the death of the auditory nerve, it would be expected that the degenerative change in all the other nerves, peripheral and central, would have a similar cause. It is, therefore, desirable that the evidence that the auditory nerve has been killed in these experimental animals because of the overgrowth of bone should be as clear as possible, and that the possibility that the bone overgrowth is secondary to degeneration of the nerve or that both bone overgrowth and nerve degeneration are independent of each other but dependent upon some third factor should be excluded.

On the whole, the evidence of the present experiment that the overgrowth of bone of the labyrinthine capsule is responsible for the death of the nerves is as clear as can probably be expected. It is hoped that further experiments in which slight degrees of change are produced will help to settle the point at issue. Even so, however, the present results demanded that the investigation should be extended to see how the other nerves of the brain and spinal cord, which are also known to degenerate under these nutritional conditions, are affected by bone overgrowth in their immediate neighbourhood.

This work is now being done and it is already clear that there is much bone hyperplasia in the neighbourhood of the optic and trigeminal nerves. Indeed the skull in immediate contact with the base of the brain,

including the bone surrounding the various foramina, is undoubtedly deformed in dogs brought up on these vitamin A-deficient diets. Early examination, therefore, supports the view that the degeneration of other nerves of the brain may well be due to the bone overgrowth as in the case of the auditory nerves. It would be expected, therefore, that a similar mechanism holds in the case of the nerves of the spinal cord, and further examination is being made on this point. It is difficult at first sight to see how mechanical factors due to bone overgrowth can destroy endogenous fibres of the central nervous system, such as those of the anterior and posterior spino-cerebellar tracts, but this point may be clarified by further investigation. In the meantime, so far as the present work goes, it seems certain that the degeneration of the auditory nerve brought about in young dogs by diets deficient in vitamin A and made up of ordinary foodstuffs is due primarily to excessive bone formation of the periosteal bone of the labyrinthine capsule and the mechanical interference of this new bone with the auditory nerve.

There remains for consideration the relation between serous labyrinthitis present in the vitamin A-deficient dogs and the bone and nerve changes above described. But little can be said of this at the present stage of the work. It is probable that serous labyrinthitis is not the earliest change, since in some dogs showing only slight abnormalities, as for instance in the animals receiving potato in their diet instead of cereal, although there is definite bone hyperplasia and early nerve degeneration, there is little or no serous labyrinthitis. In such cases albuminoid coagula are not obvious in the perilymph and the organ of Corti is normal in appearance. On the other hand, where the bone overgrowth and nerve degeneration are advanced, there is always serous labyrinthitis. It looks as if the bone and nerve changes precede the development of labyrinthitis in the earlier period of pathological change.

Another difference between the nerve degeneration and serous labyrinthitis is suggested by the appearance of the labyrinth of dog III (curative experiment). In this animal the cochlear neurones had completely disappeared, but there was no labyrinthitis. By comparison with other experimental animals it seems likely that this animal also had serous labyrinthitis at the end of the 15 weeks on a vitamin A-deficient diet. If this were the case, then the addition of vitamin A for the following 27 weeks must have cleared up the condition. It has not, however, prevented the nerve degeneration from going on to completion. Reasons have been given above for thinking that the degenerative changes in the organ of Corti and the sensory epithelium of the ampullae of the

semicircular canals are associated with the development of serous labyrinthitis and are not related to the nerve degeneration. It was pointed out, for instance, that the cochlear neurone may have completely disappeared, and yet in the absence of serous labyrinthitis the organ of Corti may appear normal or nearly normal.

Finally it may be asked, how do these vitamin A-deficient diets produce all these changes in the labyrinthine capsule? This question cannot be answered at present, but it is already clear that the answer must include not only consideration of the auditory mechanism but of the many other nerves in the body. Although it is probable that overgrowth of new bone holds the secret to the sequence of pathological changes, in the cranial nerves at least, this part of the subject has not been sufficiently investigated to allow a full description to be given, except in the case of the auditory nerve. On the other hand, a fairly complete survey of nerve degeneration produced by these diets has been worked out in the case of the dog and rabbit [Mellanby, 1934*b*, 1935], and it is now necessary to see whether all such degenerative changes in the spinal cord as well as the brain can be related directly or indirectly to overgrowth and pressure of bone. Whether this is so or not, it is already evident that overgrowth of bone in young animals due to vitamin A deficiency does not affect all bones. In the long bones the absence of vitamin A probably affects the texture of the cancellous tissue but it does not appear to alter the shape of these bones.

In view of the historical development of knowledge of bone growth, especially in regard to rickets and the discovery of the calcifying vitamin D, it is of peculiar interest that vitamin A, with which vitamin D is so often associated, should also be concerned with bone formation. The parts played by these two vitamins are, however, essentially different. In the absence of vitamin D there is overgrowth of osteoid tissue in all growing bone. In the absence of vitamin A there is overgrowth of apparently normally formed bone in certain places. In view of the growth-promoting function usually assigned to vitamin A, it is of interest to note that its presence in young dogs *prevents* overgrowth of the labyrinthine bone. It would be fruitless at this stage to conjecture the nature of the mechanism whereby vitamin A works in limiting the growth of bones at the base of the skull and other places to their normal shape, so that in its absence these bones overgrow and destroy the nerves. It is certain, however, that the explanation of this mechanism must depend on interesting physiological processes, of whose nature nothing is known at the present time.

Summary

1. The histological examination of the labyrinth capsules of young dogs fed for some months on diets of natural foodstuffs but deficient in vitamin A and rich in cereals revealed the following changes:

(*a*) degeneration of different degrees up to complete disappearance of the cochlear nerve, the cells of the spiral ganglion and their central and peripheral branches;

(*b*) degeneration, but to a lesser degree, of the vestibular division of the 8th nerve;

(*c*) overgrowth of bone in the modiolus and of the periosteal layer of the capsule near the brain.

2. This overgrowth of bone is apparently responsible for the degenerative changes in the nerves by reason of the pressing and stretching of these tissues.

3. Serous labyrinthitis also develops in the cochlea of the dogs on a vitamin A-deficient diet. This condition seems to produce degeneration of the sensory epithelium of the labyrinth, including that of the organ of Corti and of the ampullae of the semicircular canals, in course of time.

4. Substitution of potato for the cereal element of these vitamin A-deficient diets greatly reduces the abnormal changes in the labyrinth above described.

5. Examination of the base of the skull of these vitamin A-deficient dogs reveals other bone overgrowth and deformity, which is probably responsible for the degenerative changes of other cranial nerves, such as the optic and trigeminal nerves, previously described.

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EXPLANATION OF PLATES I–IV

Plate I

Fig. 1. Low-power photograph (× 10) of cochlea of dog I. Basal diet with vitamin A added. On diet 10 months. It will be seen that the internal auditory meatus is clear and the cochlear branch of the 8th nerve (*N*) has an uninterrupted path to the brain. The spiral ganglion cells (*Sp*) are clearly seen (cp. Figs. 2, etc.).

Fig. 2. Low-power photograph (× 10) of cochlea of dog II. Basal diet as for dog I, but in this case no carotene or vitamin A was added. On diet 10 months. Two new bony masses (*NB*) practically filling the internal auditory meatus can be seen. The cells of the spiral ganglion (*Sp′*) have disappeared and are replaced by connective tissue. Albuminoid coagula (*C*) can be well seen in scala tympani, indicating serous labyrinthitis.

Fig. 3. Middle whorl of cochlea (× 27) of a normal dog showing organ of Corti and cells of spiral ganglion (*Sp*).

Fig. 4. Middle whorl of cochlea (× 27) of a dog on a diet containing much cereal and deficient in vitamin A. On diet 5 months. Many cells of the spiral ganglion (*Sp′*) have disappeared together with the peripheral branches to the organ of Corti. Indications of serous labyrinthitis can be seen in the scala vestibuli.

Plate II

Fig. 5. Basal whorl of cochlea (× 27) of dog II. Diet deficient in vitamin A. On diet 10 months. The cells of the spiral ganglion (*Sp′*) have completely disappeared together with the peripheral nerve fibres to the organ of Corti. Albuminoid coagula (*C*) are obvious in both the scala tympani and scala vestibuli, indicating a condition of serous labyrinthitis. The organ of Corti is very abnormal.

Fig. 6. High-power photograph (× 266) of cells of the spiral ganglion of a normal dog. Note definite Nissl granules and well-defined nuclei.

Fig. 7. High-power photograph (× 266) of cells of the spiral ganglion of a dog whose diet was deficient in vitamin A. Nearly all the ganglion cells have completely degenerated and the few remaining cells are shrunken and the protoplasm is homogeneous. Cytoplasm is devoid of granules.

Fig. 8. Low-power photograph (×3, 2) of the cochlea and labyrinthine capsule of dog I on a diet rich in cereals and containing vitamin A. Normal or nearly normal structure.

Fig. 9*a*. Cochlea and labyrinthine capsule (×3, 2) of dog II on a diet rich in cereals and deficient in vitamin A. Not only is the internal auditory meatus nearly filled with new bone (*NB*), but the periosteal bone of the capsule, especially on the brain aspect, is greatly increased in thickness, the new bone being cancellous in type. The exit of the 8th nerve is not seen in this section.

Fig. 9*b*. The section represented here is about 2·5 mm. from that shown in Fig. 9*a*. The exit of the 8th nerve can now be seen. The two photographs (Figs. 9*a*, 9*b*) when compared with the normal (Fig. 8) illustrate the tortuous course, the compression and the stretching of the auditory nerve resulting from the bony overgrowth in dogs brought up on a vitamin A-deficient diet.

Plate III

Fig. 10. Labyrinthine capsule (×3, 7) of a normal dog, but in a plane at right angles to Figs. 8, 9*a* and 9*b*.

Fig. 11. Labyrinthine capsule (×3, 7) of a dog on a diet rich in cereals and deficient in vitamin A. There is again a mass of new bony growth in the modiolus (*NB*) and great thickening of the periosteal layer of the labyrinthine capsule, causing compression and elongation of the nerve. Compare with Fig. 10, a normal section cut in the same plane.

Fig. 12*a*. Bony overgrowth (NB^1 and NB^2) causing compression of both the cochlear and vestibular branches (*N*) of the 8th nerve in a dog on a cereal diet deficient in vitamin A (×12). Only a few degenerate cells remain in the spiral ganglion but more are found in Scarpa's ganglion (see Figs. 13, 14).

Fig. 12*b*. Cochlea (×12) of a dog maintained on a diet deficient in vitamin A for 5 months. The diet was similar to that of the previous case (Fig. 12*a*) except that potato replaced the cereal. The cells of the spiral ganglion are only slightly degenerate. There is some new bone in the modiolus (*NB*) and slight thickening of the periosteal bone. The changes seen in this animal on a potato diet are much less advanced than those of the animal on a cereal diet for the same period (Fig. 12*a*).

Plate IV

Fig. 13. Cells of Scarpa's ganglion (×266) of a normal dog.

Fig. 14. Cells of Scarpa's ganglion (×266) of a dog on a diet rich in cereals and deficient in vitamin A (see Fig. 12*a*). Although the cells are comparatively normal in structure, they are markedly elongated, apparently due to the pressure of the new bone (*NB*) shown in Fig. 12*a*. These cells seem very resistant to mechanical injury as compared with those of the spiral ganglion.

Figs. 15*a* and 15*b*. Low-power (×27) and high-power (×300) photographs of ampulla of dog I on a diet rich in cereals and vitamin A. The epithelium is thin, there is good differentiation between nuclei and cytoplasm of the cells and the hair-like projections are clearly seen.

Figs. 16*a* and 16*b*. Low-power (×27) and high-power (×300) photographs of ampulla of dog II on a cereal diet deficient in vitamin A. The epithelium is thickened, the differentiation between nuclei and cytoplasm is poor, and the hairy projections have in some places disappeared.